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Characterisation of a "European-Like" Serotype G8 Human Rotavirus Isolated in Australia

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An atypical human rotavirus strain, DG8, was isolated from a 13-month-old child hospitalised with acute gastro-enteritis in Australia. The virus could not be serotyped by enzyme immunoassay (EIA) using standard reagents specific for common Group A human rotavirus G serotypes. The deduced amino acid sequence of the outer capsid glycoprotein, VP7, indicated that this strain belonged to the uncommon human serotype G8. This was confirmed by EIA incorporating a G8-specific neutralising monoclonal antibody (NMAb). The VP4 genotype of DG8 was determined as P[14], equivalent to P serotype P3B, by sequence analysis and confirmed by EIA incorporating a P3B-specific NMAb. Electrophoretic analysis of DG8 genomic dsRNA indicated that the virus exhibited a "long" electropherotype. Northern hybridisation analysis (using a whole-genome probe derived from DG8) indicated that DG8 shared overall homology with the European serotype G8 strain, HAL1166 (11 of 11 genes). In contrast, only 9 of 11 genes of DG8 hybridised with the Asian serotype G8 strain, B37, and with the bovine G8 strain, A5. Hence, DG8 displayed features reminiscent of the human serotype G8 rotaviruses isolated in Europe in the mid-1980s rather than the geographically local G8 Asian strains isolated a decade earlier. It is possible that DG8 arose through reassortment between human and bovine rotaviruses. ***J. Med. Virol. 60:56–62, 2000.***

KEY WORDS: epidemiology; viral antigens; genetic reassortment

INTRODUCTION

Group A rotaviruses are the major cause of acute gastro-enteritis in infants and young children worldwide [Kapikian and Chanock, 1996]. Rotaviruses are classified into G and P serotypes according to the type of VP7 and VP4 neutralisation antigens, respectively, present on the outer capsid of the virus [Estes, 1996]. Of the 14 G-types defined currently, most strains causing infection requiring hospital admission are classified into serotypes G1-4, which are the target of the first licensed human vaccine. However, infection by G6, 8, 9, 10, and 12 viruses have been reported [Estes, 1996] albeit at low frequencies. Future vaccines might require the incorporation of these less common serotypes should continued surveillance find them to be of epidemiological importance.

Serotype G8 rotaviruses were first isolated from children with acute gastro-enteritis in Indonesia [Hasegawa et al., 1984; Matsuno et al., 1985; Albert et al., 1987]. These strains exhibited a distinct and unique electrophoretic migration pattern of the 11 genomic RNA segments that was designated the "super-short" electropherotype. Subsequently isolated serotype G8 strains from Finnish and Italian children displayed a more typical "long" electropherotype [Gerna et al., 1990b]. Recently, serotype G8 viruses with unusual serotypic properties were identified in Africa [Cunliffe et al., 1999]. These observations suggested that distinct populations of human serotype G8 rotaviruses might have circulated in different geographical locations. Serotype G8 strains have also been isolated from cattle [Snodgrass et al., 1990; Taniguchi et al., 1991], pigs [Gouvea et al., 1994] and a horse [Isa et al., 1996].

In this report, the genetic and antigenic characteristics are described of a human serotype G8 rotavirus isolated from a child admitted to the Royal Darwin Hospital in northern Australia. This analysis included the determination of the sequences of the VP7 and VP4 genes and the reactivities of the virus to G and P type specific neutralising monoclonal antibodies (NMAbs). The strain, designated DG8 (for Darwin G-type 8), was found to be closely related to the geographically remote European G8 strain HAL1166 rather than to the local Asian viruses and may have arisen through interspecies genetic reassortment.

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MATERIALS AND METHODS

Viruses Used in This Study

Strain DG8 was isolated from a 13-month-old child admitted to hospital with acute gastro-enteritis in January, 1996. The isolate was tested by routine enzyme immunoassay (EIA) and was typed as subgroup I (SGI) but was non-reactive with G1-, 2-, 3-, and 4-specific NMAbs. Electrophoretic analysis of DG8 RNA indicated that it exhibited a long electropherotype. The virus was adapted to growth in MA104 cells in the presence of 1 μg/ml porcine trypsin (Sigma, St. Louis, MO) and partially purified by fluorocarbon extraction and ultracentrifugation. Standard tissue culture adapted rotavirus strains used in this study and their relevant G type and P type (serotype[genotype]) designations are as follows: RV4 (G1P1A[8]), Wa (G1P1A[8]), M37 (G1P2[6]), RV5 (G2P1B[4]), S2 (G2P1B[4]), 1076 (G2P2[6]), RV3 (G3P2[6]), P (G3P1A[8]), ST3 (G4P2[6]), PA169 (G6P3B[14]), MG6 (G6P3B[13]), B37 (G8), HAL1166 (G8P3B[14]), A5 (G8P6[1]), and F45 (G9P1A[8]). G and P type designations are from Estes [1996]. MG6 was described previously [Palombo and Bishop, 1995]. The viruses were grown in MA104 cells and purified as described above.

Extraction of Double-Stranded RNA, Reverse Transcription-Polymerase Chain Reaction (RT-PCR), and Nucleotide Sequence Determination

Double-stranded genomic RNA was isolated by phenol-chloroform extraction of virus followed by purification by adsorption to hydroxyapatite [Gouvea et al., 1991]. RNA was electrophoresed in 10% (w/v) polyacrylamide gels and stained with silver nitrate [Dyall-Smith and Holmes, 1984]. Complementary DNA of gene segments encoding VP7 and the VP8* subunit of VP4 was synthesised and amplified by the coupled RT-PCR methods of Gouvea et al. [1990] and Gentsch et al. [1992]. The cDNAs were gel purified and the sequences of the VP7 and VP8* genes were determined directly using the fmol DNA Sequencing System (Promega, Madison, WI) and gene-specific primers. The nucleotide sequences have been deposited in the GenBank database and assigned the accession numbers AF034852 (VP7 gene) and AF034853 (VP8* gene).

Determination of G and P Types by EIA

G typing using the G8 specific NMAb B37:1 [Tursi et al., 1987], reacted at a dilution of 1:32,000, and P typing using the P3B[14]-specific NMAb 2H4 [Gerna et al., 1994a], reacted at a dilution of 1:2,000, were carried out as described previously [Coulson et al., 1987; Masendycz et al., 1997]. Standard viruses of defined G and P types were included in the analysis.

Determination of G and P Types by RT-PCR

G typing was carried out using the method described by Gouvea et al. [1990] using G1-, 2-, 3-, 4-, 8-, and 9-specific primers. P typing was carried out according to the method of Gentsch et al. [1992] using P1A[8]-, 1B[4]-, 2[6]-, 3[9]-, and 4[10]-specific primers.

Northern Hybridisation Analysis

Whole genome probe of DG8 dsRNA was prepared by labelling with digoxigenin (DIG) by chemical linking of DIG to RNA using the DIG Chem-Link reagent (Roche Biochemicals, Germany). Northern hybridisation and detection of bound probe was carried out as described previously [Palombo et al., 1996].

RESULTS

Determination of G Type of DG8 and Sequence of the VP7 Protein

Strain DG8 was found to be non-reactive to NMAbs specific for serotypes G1, G2, G3, and G4 in EIA. An RT-PCR based method was therefore used in an attempt to determine the G type of the strain. Full-length VP7 cDNA (1,062 bp) of DG8 was derived by RT-PCR using common VP7 gene end primers. The cDNA was used in the nested PCR typing assay of Gouvea et al. [1990] using a series of G type specific primers; however, the assay failed to generate products of expected sizes. This failure raised the possibility that DG8 belonged to an uncommon human G serotype. The VP7 gene was therefore sequenced and the deduced amino acid sequence of VP7 was determined (Fig. 1). This protein exhibited 94.4–97.5% amino acid identity to serotype G8 viruses from human (HAL1166, B37, and 69M) and animal (A5 and 678) origins. Sequence comparisons of VP7 have indicated that strains of the same G type exhibit > 91% amino acid identity [Kapikian and Chanock, 1996]. By this criterion, DG8 appeared to belong to serotype G8. Importantly, the three established antigenic regions of VP7, i.e., regions A (amino acids 87–101), B (aa142–152), and C (aa208–221) [Estes, 1996; Kapikian and Chanock, 1996] were almost completely conserved between DG8 and the other serotype G8 strains. The VP7 of DG8 was most closely related to that of HAL1166 (97.5% identity), a human strain isolated in Finland in 1986 [Gerna et al., 1990b] and most distantly related to B37 (94.4% identity), a human "super-short" strain from Indonesia which was isolated in 1978–1979 [Albert et al., 1987].

The nucleotide sequence of the DG8 VP7 gene may explain why this strain was not reactive in the nested PCR typing assay. Examination of the binding site of the serotype G8 specific primer (aAT8) included in this assay indicated that a single base mismatch occurred between DG8 cDNA and the 3′ terminal nucleotide of aAT8. Hence, no PCR product would have been generated even though the sequence of the primer otherwise matched that of DG8.

To confirm the genetic classification of DG8 as serotype G8, this strain was tested for its ability to react with the G8-specific NMAb, B37:1, in an EIA (Table I). This analysis confirmed that DG8 exhibited the antigenic properties of a serotype G8 rotavirus.

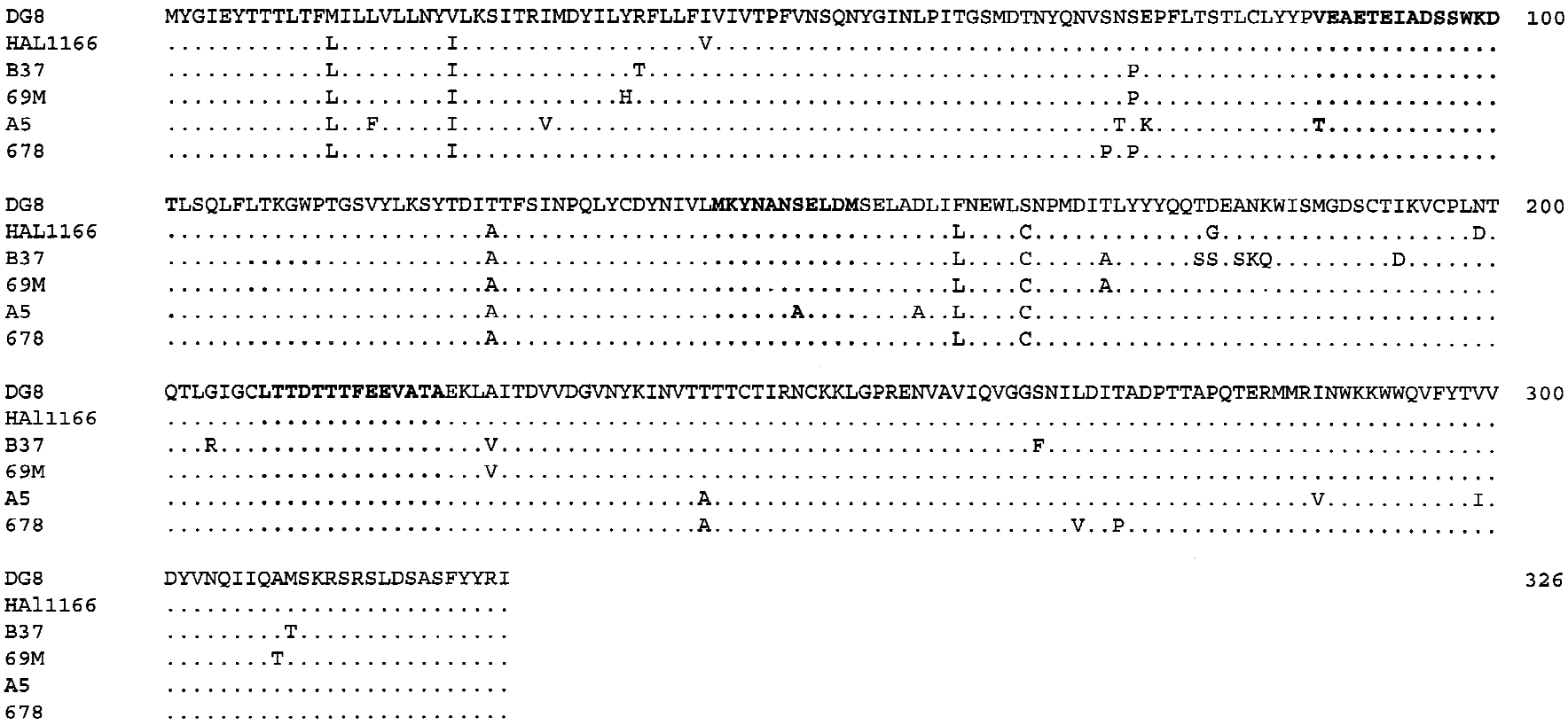

Fig. 1. Deduced amino acid sequences of VP7 from DG8 and human (HAL1166, B37, 69M) and bovine (A5, 678) serotype G8 strains. Dots indicate residues identical to DG8. Residues in bold face indicate the antigenic regions A (amino acids 87–101), B (aa142–152), and C (aa208–221). Sequences for HAL1166, B37, 69M, A5, and 678 were obtained from Green et al. [1989], Hum et al. [1989], Taniguchi et al. [1991], and Gerna et al. [1994b].

TABLE I. Reactivity of Human Rotavirus Strains With the Serotype G8-specific, NMAb, B37:1, and the Serotype P3B-Specific NMAb, 2H4, in EIA*

Strain	G type	P serotype [genotype]	P/N value[a] with NMAb B37:1	P/N value[a] with NMAb 2H4
RV4	1	1A[8]	1.1	1.5
Wa	1	1A[8]	1.0	1.3
M37	1	2[6]	1.0	1.4
RV5	2	1B[4]	1.1	**4.4**
S2	2	1B[4]	1.2	**5.5**
1076	2	2[6]	1.1	**3.4**
RV3	3	2[6]	1.0	1.5
P	3	1A[8]	1.9	NT
ST3	4	2[6]	1.1	**2.8**
PA169	6	3B[14]	1.0	**5.7**
MG6	6	3B[14]	NT	**2.7**
B37	8	ND	**15.8**	1.2
HAL1166	8	3B[14]	**23.0**	**7.2**
F45	9	1A[8]	1.0	NT
DG8	?	?	**28.0**	**8.9**

*NMAb, neutralizing monoclonal antibody; EIA, enzyme immunoassay; NT, not tested; ND, not defined.
[a]A virus was considered positive if its A450 with B37:1 or 2H4 using homologous polyclonal antibody as the coating antibody (P value) was at least twice the A450 obtained with preimmune immunoglobulins (N value), i.e., a P/N value of ≥2.0. Positive values are in bold face.

Determination of P Type of DG8 and Sequence of the VP8* Protein

The RT-PCR P typing assay of Gentsch et al. [1992] was used to determine the P genotype of DG8. Complementary DNA (887 bp) of the VP8* trypsin cleavage subunit of VP4, which contains serotype specific neutralisation epitopes of VP4 [Larralde and Gorziglia, 1992; Padilla-Noriega et al., 1995; Kirkwood et al., 1996], was generated and used in nested PCR with P-type-specific primers. This assay did not produce any PCR products of expected sizes, suggesting that DG8 belonged to an uncommon P type. The VP8* cDNA was therefore sequenced and the deduced amino acid sequence was determined (Fig. 2). This protein was found to exhibit 94.6–96.6% amino acid identity (to the first trypsin cleavage site) with the VP8* from standard strains belonging to the uncommon P genotype 14 (P serotype 3B). According to VP4 sequence comparisons, strains of the same P type have been shown to exhibit > 89% amino acid identity [Estes, 1996; Kapikian and Chanock, 1996]. Hence, DG8 was genetically related to P3B[14] viruses. This serotype includes human strains exclusively, some of which are believed to have derived many of their genes from bovine viruses [Gerna et al., 1990a]. Only one of these strains, MG6, was isolated in Australia [Palombo and Bishop, 1995], whereas the other two were European in origin [Gerna et al., 1990a, 1990b].

The antigenic properties of the DG8 VP4 were investigated by testing its ability to react with the P3B[14]-specific NMAb, 2H4, in an EIA (Table I). Although this NMAb displayed some level of cross-reactivity with P1B[4] and some P2[6] standard strains, the greatest level of reactivity was generally observed with viruses classified as P3B[14]. DG8 displayed a strong level of reactivity with 2H4, thus confirming its classification into P serotype 3B as suggested from the sequence data. The cross-reactivity of 2H4 in EIA may be mediated by structural similarity of the epitope recognised by this NMAb in viruses of different VP4 types. However, the binding site of 2H4 has not been identified, so this possibility cannot be investigated by sequence data analysis.

Electrophoretic and Northern Hybridisation Analysis of DG8 dsRNA

Double-stranded RNA extracted from DG8 was electrophoresed in a 10% (w/v) polyacrylamide gel and compared with dsRNA from HAL1166 because this virus had been shown to exhibit a long electropherotype. DG8 and HAL1166 displayed similar long patterns (Fig. 3). However, co-electrophoresis of RNA from DG8 and HAL1166 indicated that at least six segments (segments 1, 2, 5, 7, 9, and 10) exhibited obvious mobility differences (data not shown).

Northern hybridisation analysis, using whole genome probe derived from DG8, indicated that DG8 and HAL1166 shared overall genetic homology, with all 11 gene segments cross-hybridising (Fig. 3). In contrast, genes 4 and 5 of B37 and the bovine strain A5 (isolated in Thailand) exhibited a marked reduction in signal compared with the cognate genes of DG8 and HAL1166 (Fig. 3).

DISCUSSION

Genetic and antigenic analysis of an atypical rotavirus strain responsible for acute gastro-enteritis in a 13-month-old child indicated that this virus could be classified as G8P3B[14]. This is the first documented description of a serotype G8 rotavirus, and the second of a virus belonging to P type 3B[14], in Australia.

The identification of serotype G8 strains in epidemiological surveys of rotaviruses from children hospitalised with severe gastro-enteritis is uncommon. Where the incidence of serotype G8 viruses has been investigated, these surveys have shown that this serotype was absent [Nakagomi et al., 1991; Steele et al., 1995] or negligible [Noel et al., 1994; Beards and Graham, 1995]. A recent study, in contrast, indicated a high proportion (more than 50% of isolates tested) of G8 isolates in Malawi, suggesting that this serotype may be more common than previously appreciated [Cunliffe et al., 1999]. This finding is supported by serological surveys that indicated antibody to serotype G8 was detectable in a high proportion of children and adults [Brussow and Sidoti, 1991; Kelkar et al., 1996], suggesting that these viruses circulated in various communities. It is possible that serotype G8 rotaviruses may commonly infect children but only rarely result in severe disease requiring hospitalisation. This effect might be mediated through cross-protection by other rotavirus serotypes. The region C of the VP7 of serotype G8 human strains is similar to that of viruses belonging to the more common serotype G3 [Hum et al., 1989]. Hence, some level of protection may be occurring through prior

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DG8       MASPFYRQLLSNSYVTNISDEVSEIGTRKATNVTVNPGPFAQRGYAPVNW   50
HAL1166   ...LI.....................A..T...........T.......
MG6       ..........................A..............T.......
PA169     ...LI.....................A..T...........T.......

DG8       GHGELSDSTLVQPTIDGPYQPTTFNLPIDYWMLIAPTQIGRVAEGTNTTD  100
HAL1166   ..............L.................................
MG6       ..............L.................................
PA169     ..............L.................................

DG8       RWFASVLVEPNVSNTQREYVLDGQTVQLQVSNDSSTLWKFILFIKLEKNG 150
HAL1166   ....C.......Q...................N.................
MG6       ....C.........................A..S.................
PA169     ....C.............................N.................

DG8       TYSQYSTLSTSNKLCAWMKREGRVYWYAGTTPNASESYYLTINNDNSNVS 200
HAL1166   A...............................................
MG6       ...............................................
PA169     ...............................................

DG8       CDAEFYLIPRSQTDLCAQYINNGLPPIQNTRNVVPVSIASR↓EIRHTR↓  247
HAL1166   .............E..T.....................T.. ......
MG6       .............E.........................V.. ......
PA169     .............E.........................S.. ......
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Fig. 2. Deduced amino acid sequences of VP8* from DG8 and human P3B[14] strains HAL1166, MG6, and PA169. Dots indicate residues identical to DG8, and the arrows after amino acids 241 and 247 indicate the sites of trypsin cleavage of VP4. Sequences for HAL1166, MG6, and PA169 were obtained from Gerna et al. [1994a] and Palombo and Bishop [1995].

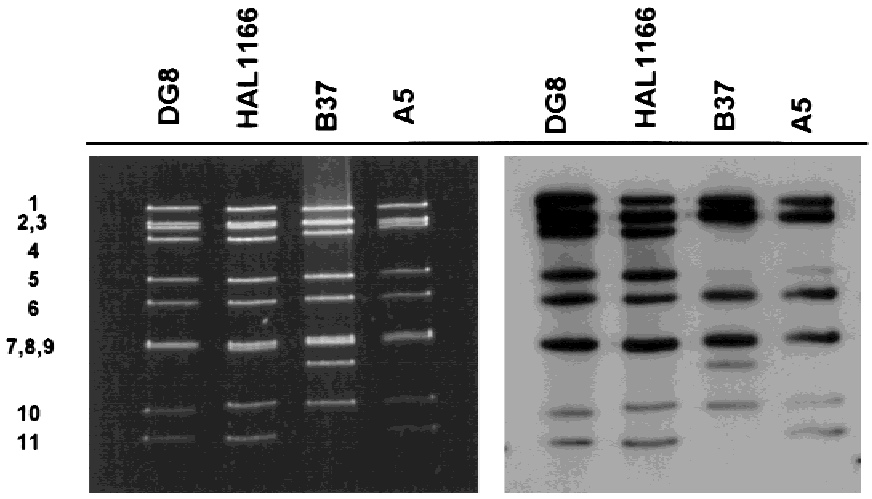

Fig. 3. Northern hybridisation analysis of human (DG8, HAL1166, and B37) and bovine (A5) serotype G8 rotavirus strains using a whole genome probe derived from DG8. RNA on the left panel was stained with ethidium bromide; the right panel shows gene segments hybridising with the DG8 probe.

infection with serotype G3 strains circulating in the paediatric community.

The unusual characteristics of DG8 allow speculation about its possible origins. The genetic and antigenic similarity between DG8 and HAL1166 suggested that the local serotype G8 virus might be derived from a European strain. This relationship was emphasised by the high level of VP7 identity observed between DG8 and HAL1166. Both these viruses exhibited long RNA electropherotypes, whereas the geographically local Asian serotype G8 human viruses displayed a unique "super-short" electropherotype. However, bovine serotype G8 strains exhibit long electropherotypes [Taniguchi et al., 1991], raising the possibility that DG8 might be derived from these or other local bovine viruses. In support of this possibility is the finding that some human serotype G8 viruses have been shown, by RNA-RNA hybridisation, to be related to bovine rotaviruses [Oshima et al., 1990; Browning et al., 1992]. This finding is also supported by our Northern hybridisation results showing that the DG8 genome is related to the bovine strain A5, with the exception of genes 4 and 5. These genes encode proteins (VP4 and NSP1, respectively) implicated in host range restriction and virulence [Burke and Desselberger, 1996]. Hence, it is possible that DG8 (and HAL1166) was derived from reassortment events combing a bovine gene constellation with VP4 and NSP1 genes that allowed replication in humans. We have previously described the human serotype G6 strain MG6, which contains a P3B[14]-type VP4 [Palombo and Bishop, 1995], the same type present in DG8. Human serotype G6 viruses are believed to be derived from bovine strains that have acquired a novel VP4 gene. Hence, DG8 may have developed locally from a bovine genetic background by reassortment with an MG6-like virus, or a common progenitor, into a strain that resembles the European human serotype G8 viruses.

The identification and characterisation of a previously absent serotype in the Australian community indicates that surveillance of rotavirus strains is necessary to monitor the emergence of novel serotypes into the wild-type population. This is especially important in the context of future vaccine development. Continued genetic analysis of rotavirus isolates and additional analysis of DG8 and similar strains might give further insights of the role the introduction of animal genes into the human virus gene pool plays in the evolution of the rotavirus population.

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